

MUTATION UPDATE

Activating and Inactivating Mutations in the Human GNAS1 Gene

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Communicated by Christopher G. Mathew

GNAS1 on chromosome 20 is a complex locus, encoding multiple proteins, of which G_sα, the α-subunit of the heterotrimeric stimulatory G protein G_s, is of particular interest clinically. Amino acid substitutions at two specific codons lead to constitutive activation of G_sα. Such gain-of-function mutations are found in a variety of sporadic endocrine tumors and in McCune-Albright syndrome, a sporadic condition characterized by multiple endocrine abnormalities. Heterozygous loss of G_sα function results in the dominantly inherited condition, Albright hereditary osteodystrophy (AHO). Here we present a review of published GNAS1 mutations and report 19 additional mutations, of which 15 are novel. A diverse range of inactivating mutations has been detected, scattered throughout the gene but showing some evidence of clustering. Only one, a recurring 4 bp deletion in exon 7, could be considered common among AHO patients. The parental origin of the mutation apparently determines whether or not the patient shows end-organ resistance to hormones such as parathyroid hormone. G_sα is biallelically expressed in all tissues studied to date and thus there is no direct evidence that this transcript is imprinted. However, the recent identification of other imprinted transcripts encoded by GNAS1 and overlapping G_sα, together with at least one imprinted antisense transcript, raises intriguing questions about how the primary effect of mutations in GNAS1 might be modulated. *Hum Mutat* 16:183–189, 2000. © 2000 Wiley-Liss, Inc.

KEY WORDS: GNAS1; G_s-alpha; McCune-Albright syndrome; MAS; Albright hereditary osteodystrophy; AHO; pseudohypoparathyroidism

DATABASES:

GNAS1 – OMIM:139320, 174800 (MAS), 103580 (AHO); GDB:120628; GenBank:NM_000516; HGMD:GNAS1; <http://www.le.ac.uk/genetics/maa7/GNAS1/>

BACKGROUND

GNAS1 is one of only a few genes in which both activating and inactivating mutations have been characterized (MIM# 139320). Located on chromosome 20q13, GNAS1 was originally described as a gene comprising 13 exons that encode G_sα, the α-subunit of the heterotrimeric stimulatory G protein G_s [Kozasa et al., 1988]. More recently, additional exons have been identified and it has become apparent that GNAS1 is a complex locus encoding multiple overlapping transcripts [Hayward et al., 1998a, b]. Of these, G_sα is the most extensively characterized and clinically relevant. In this review we therefore focus on mutations in exons 1–13 of GNAS1 and their effects on G_sα.

G proteins mediate signal transduction across

cell membranes, coupling extracellular receptors to intracellular effector proteins such as ion channels and the adenylyl cyclase and phospholipase C second messenger systems. They consist of alpha, beta, and gamma subunits, each encoded by different genes. The α-subunit contains the guanine nucleotide-binding site, has intrinsic GTPase activity, and confers receptor specificity. GNAS1 shows a high degree of sequence conservation

Received 1 February 2000; accepted revised manuscript 22 May 2000.

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Contract grant sponsor: Research Trust for Metabolic Diseases of Childhood.

across species. In addition, there are regions of significant amino acid conservation between $G_s\alpha$ and other G protein α -subunits.

G_s couples multiple hormones, including parathyroid hormone (PTH), thyroid stimulating hormone (TSH), and luteinizing hormone (LH), to adenylyl cyclase. There are four primary $G_s\alpha$ transcripts, resulting from the combinatorial effects of two alternative splicing events [Bray et al., 1986; Kozasa et al., 1988]. The full-length transcript produces a 52kDa protein (isoforms 1 and 2), while alternative splicing of exon 3 gives rise to a shortened 45kDa isoform (isoforms 3 and 4). Exon 3 encodes a short hydrophilic region that is not common to other G protein α -subunits. In addition, the use of an alternative splice acceptor site for exon 4, 3 bp upstream, results in insertion of a serine residue in isoforms 2 and 4. $G_s\alpha$ is expressed in a wide range of tissues. However, the distribution of the different isoforms varies. For instance, the 52kDa isoforms are not seen in erythrocyte membranes [see Miric et al., 1993]. Nomenclature for mutations described in this review refers to the sequence of isoform 1 [Kozasa et al., 1988] (Genbank accession no. NM_000516).

Gain-of-Function Mutations

Specific amino acid substitutions occurring at codons 201 and 227 confer a gain-of-function on $G_s\alpha$. Arg²⁰¹ is in the GDP/GTP binding domain of the protein and is ADP-ribosylated in the presence of cholera toxin, while Gln²²⁷ is located in the G4 domain required for the intrinsic GTPase activity of $G_s\alpha$. Replacement of either amino acid results in constitutive activation of $G_s\alpha$ and hence of adenylyl cyclase. Such mutations were first characterized in pituitary tumors that secreted human growth hormone and showed increased adenylyl cyclase activity [Landis et al., 1989]. They have since also been identified in a variety of other endocrine tumors, including pituitary adenomas, thyroid adenomas and carcinomas, parathyroid adenomas, and pheochromocytomas [Lyons et al., 1990; reviewed by Ringel et al., 1996]. In these mutated forms, *GNAS1* is known as the *gsp* oncogene.

Activating mutations at codon 201 can also lead to a condition known as McCune Albright syndrome (MAS; MIM# 174800) [Weinstein et al., 1991; Schwindinger et al., 1992; Riminucci et al., 1999]. MAS is characterized by polyostotic fibrous dysplasia, café au lait patches on the skin, and endocrine anomalies such as hyperthyroidism and precocious puberty. Individuals with MAS are somatic mosaics, presumably as a result of mutation

during embryonic development, and the mutation is most readily detectable in the affected tissues [Weinstein et al., 1991; Schwindinger et al., 1992]. Germ-line transmission of these activating mutations has not been observed, suggesting that they are likely to be embryonic lethal if present in non-mosaic form [Ringel et al., 1996].

Loss-of-Function Mutations

Heterozygous inactivating mutations of $G_s\alpha$ result in a condition known as Albright Hereditary Osteodystrophy (AHO; MIM# 103580). This is an autosomal dominant disorder characterized by short stature, round face with a low, flat nasal bridge, shortening of the fourth and fifth metacarpals and metatarsals, obesity, subcutaneous ossification, intracranial calcification, and variable degrees of mental retardation [reviewed by Wilson and Trembath, 1994]. Patients may show end-organ resistance to multiple hormones including PTH and TSH, a condition known as pseudohypoparathyroidism type Ia (PHPIa). This may lead to a low serum calcium, and elevated levels of phosphate, PTH, and TSH. Other patients with AHO have a normal biochemical profile or pseudopseudohypoparathyroidism (PPHP). The PHPIa and PPHP phenotypes have been observed to segregate within the same family and thus represent variable expressivity of the same mutation. All such patients show a 50% reduction in $G_s\alpha$ activity in all tissues studied when compared to normal controls. There is an apparent parent-of-origin effect, maternal transmissions usually leading to the PHPIa phenotype, while mutations of paternal origin result in PPHP [Davies and Hughes, 1993; Wilson et al., 1994]. However, $G_s\alpha$ is biallelically expressed in all tissues studied and thus there is no direct evidence that this transcript is imprinted [Campbell et al., 1994].

Mutations observed in AHO patients are distributed throughout the gene. Small insertions/deletions and amino acid substitutions predominate, but nonsense mutations and point mutations that lead to altered translation initiation or aberrant mRNA splicing have also been documented (see Table 1). The most common mutation is a 4 bp deletion in exon 7 that has been reported in 11 families worldwide. In several cases it has been demonstrated to have arisen de novo [Yu et al., 1995; this study]. Thus, as might be expected in a sometimes severe autosomal dominant condition, this deletion represents a recurring new mutation rather than a founder effect.

The majority of other mutations are unique to

TABLE 1. Mutations and Polymorphisms in the Human GNAS1 Gene[†]

Category	Exon	Mutation ^a	Amino acid ^a	Mutation type	Comment	Reference
LOF	01	c.1A>G		Translation initiation	Familial	Patten et al., 1990
LOF	01	c.1A>G		Translation initiation		This study
LOF	01	c.91C>T	Q31X	Nonsense	Familial	de Beur et al., 1998**
LOF	01	c.111C>G	Y37X	Nonsense	Familial	de Beur et al., 1998**
LOF	01	c.115delG		Frameshift	Sporadic de novo	This study
LOF	01	c.119-139+17del38		Frameshift	Familial	Fischer et al., 1998
LOF	01	c.124C>T	R42C	Missense	Familial	This study
LOF	02	c.144-145insT		Frameshift	Sporadic de novo	This study
LOF	04	c.275T>A	V92E	Missense	Familial	This study
LOF	04	c.275-312+8del43		Frameshift	Sporadic de novo	Oude Luttikhuis et al., 1994
LOF	04	c.296T>C	L99P	Missense	Familial	Miric et al., 1993
LOF	04	c.301-302delGA		Frameshift	Familial	Yu et al., 1999
LOF	04	c.308T>C	I103T	Missense	Familial	This study
LOF	05	c.343C>T	P115S	Missense	Sporadic de novo	Ahmed et al., 1998
LOF	05	c.348delC		Frameshift		Shapira et al., 1996
LOF	05	c.348delC		Frameshift		This study
LOF	05	c.395T>C	L132P	Missense	Familial	This study
LOF	05	c.432+1G>A		Splice donor	Sporadic de novo	Wilson et al., 1994
LOF	05	c.432+1G>A		Splice donor		This study
LOF	06	c.473-474insG		Frameshift	Sporadic de novo	This study
LOF	06	c.478C>T	R160C	Missense	Familial	This study
LOF	06	c.489C>G	Y163X	Nonsense		This study
LOF	06	c.493C>T	R165C	Missense	Familial	Miric et al., 1993
LOF	07	c.565-568delGACT		Frameshift		Weinstein et al., 1992
LOF	07	c.565-568delGACT		Frameshift	Sporadic de novo	Yu et al., 1995
LOF	07	c.565-568delGACT		Frameshift	Familial	Yu et al., 1995
LOF	07	c.565-568delGACT		Frameshift		Yu et al., 1995
LOF	07	c.565-568delGACT		Frameshift		Yu et al., 1995
LOF	07	c.565-568delGACT		Frameshift	Familial	Yokoyama et al., 1996
LOF	07	c.565-568delGACT		Frameshift	Familial	Ahmed et al., 1998
LOF	07	c.565-568delGACT		Frameshift	Familial	Ahmed et al., 1998
LOF	07	c.565-568delGACT		Frameshift	Familial	Nakamoto et al., 1998
LOF	07	c.565-568delGACT		Frameshift	Familial	Walden et al., 1999
LOF	07	c.565-568delGACT		Frameshift	Sporadic de novo	This study
LOF	08	c.617-618delGA		Frameshift	Familial	Yu et al., 1999
LOF	08	c.640-643delGTGG		Frameshift	Familial	Miric et al., 1993
LOF	09	c.692G>A	R231H	Missense	Familial	Farfel et al., 1996
LOF	10	c.750C>G	S250R	Missense		Warner et al., 1997
LOF	10	c.772C>T	R258W	Missense	Sporadic de novo	Warner et al., 1998
LOF	10	c.776A>T	E259V	Missense	Sporadic de novo	Ahmed et al., 1998
LOF	10	c.790A>C	N264H	Missense		This study
LOF	10	c.799-800insC		Frameshift		Shapira et al., 1996
LOF	10	c.814delC		Frameshift	Familial	Weinstein et al., 1990
LOF	10	c.839+1G>C		Splice donor	Familial	Weinstein et al., 1990
LOF	11	c.845T>C	L282P	Missense	Sporadic de novo	This study

(continued)

TABLE 1. (Continued)

Category	Exon	Mutation ^a	Amino acid ^a	Mutation type	Comment	Reference
LOF	11	c.909-910insT		Frameshift		This study
LOF	13	c.1106-1107delCT		Frameshift		This study
LOF	13	c.1107-1108delTG		Frameshift		This study
LOF	13	c.1107-1108ins12	369-370ins AVDT	In-frame insertion	Familial de novo, germ-line mosaicism	Aldred et al., in press
LOF	13	c.1154G>A	R385H	Missense		Schwindinger et al., 1994
LOF _{ts} -GOF*	13	c.1096G>T	A366S	Missense	AHO & testotoxicosis	Nakamoto et al., 1993
LOF _{ts} -GOF*	13	c.1096G>T	A366S	Missense	AHO & testotoxicosis	Nakamoto et al., 1993
GOF	08	c.601C>T	R201C	Missense	Mc-Cune Albright & endocrine tumours	Landis et al., 1989; Lyons et al., 1990; Weinstein et al., 1991; Yang et al., 1996
GOF	08	c.601C>G	R201G	Missense	Mc-Cune Albright	Riminucci et al., 1999
GOF	08	c.601C>A	R201S	Missense	Endocrine tumour	Yang et al., 1996
GOF	08	c.602G>A	R201H	Missense	Mc-Cune Albright & endocrine tumours	Landis et al., 1989; Lyons et al., 1990; Weinstein et al., 1991; Schwindinger et al., 1992
GOF	09	c.680A>G	Q227R	Missense	Endocrine tumours	Landis et al., 1989; Lyons et al., 1990
GOF	09	c.681G>CorT	Q227H	Missense	Endocrine tumour	Lyons et al., 1990
Pm	05	c.393T>C	I131I	Polymorphism	FokI RFLP	Miric et al., 1993
Pm	07	c.555C>T	I185I	Polymorphism	BclI RFLP	This study
Pm	13	c.1113C>T	N371N	Polymorphism	FokI RFLP	Waltman et al., 1994

[†]Mutations and polymorphisms from the literature are reviewed, together with our own data generated from diagnostic service analysis. Mutations in intronic sequences are grouped with the nearest exon. The presence of a family history is documented where known and “sporadic de novo” denotes cases where a new mutation has been confirmed by testing of both parents.

^aNovel sequence changes reported in this study are highlighted in bold.

LOF, loss of function; GOF, gain of function; ts, temperature sensitive; pm, polymorphism; *, the gain-of-function phenotype is not predicted to occur in females; **, an additional mutation (Q21X) reported by these authors has been omitted because the codon number and base substitution are inconsistent.

individual families, but some clustering is apparent, particularly in exons 1, 4, 5, 10, and 13 (see Fig. 1). We have identified three different insertion/deletions clustered at nucleotides 1106–1108 in exon 13, implicating this as a potential mutation hot-spot. Mutations of exon 1 are under-represented in the literature, as the extremely GC-rich nature of the flanking sequences has precluded its analysis by many authors. However, in one study, mutations in exon 1 accounted for 26% (7/27) of the total identified [Oude Luttikhuis, 1996]. No mutations have yet been found in exon 3. This is perhaps not surprising given the alternative splicing patterns observed and the lack of conservation with other α -subunits. Mutations within exon 3 might therefore have little or no clinical consequence.

An intriguing mutation, A366S, has been reported in two unrelated male patients affected with PHP1a and testotoxicosis [Nakamoto et al., 1993]. Testotoxicosis is a condition in which secretion of testosterone by the Leydig cells in the absence of luteinizing hormone leads to precocious puberty and is part of the spectrum of endocrine disorders seen in McCune-Albright syndrome. What could explain the paradoxical coexistence of both activating and inactivating phenotypic features in the same patient? Iiri et al. [1994] demonstrated that A366S leads to constitutive activation of adenylyl cyclase by causing accelerated release of GDP, thus increasing the fraction of active GTP-bound $G_s\alpha$. However, while this mutant protein was stable at the reduced temperature of the testis, it was thermolabile at 37°C. In tissues other than the testis, the mutation therefore resulted in reduced $G_s\alpha$ activity and an AHO phenotype. The mutation has not been observed in females but it would be expected to behave as any other inactivating mutation and only give rise to AHO.

Novel Mutations

We have identified mutations in 19 previously unreported AHO patients referred to us for diagnostic testing. Mutation analysis was carried out by single stranded conformation analysis and direct sequencing. All variants were confirmed by sequencing of a second independent PCR product. The likely pathogenic nature of missense mutations was confirmed by their absence in 50 normal individuals. Segregation within affected families or the de novo occurrence of a mutation in sporadic cases was demonstrated where family material was available. Of the 19 mutations, 15 are novel (see Table 1). We have also documented the presence of germline mosaicism for a mutation in one family [Aldred et al., in press]. A locus-specific database for GNAS1 is now under development at <http://www.le.ac.uk/genetics/maa7/GNAS1>.

Polymorphisms and Other Non-pathogenic Mutations

An intragenic T>C polymorphism in exon 5 creates a *FokI* restriction fragment length polymorphism that has been useful both in linkage analysis and in studying parental origin of GNAS1 transcripts. Heterozygosity at this RFLP approaches 50% [Miric et al., 1993] but another *FokI* RFLP in exon 13 is much less informative [Waltman et al., 1994]. We have observed an additional single nucleotide polymorphism in exon 7 that abolishes a *BclI* restriction site (see Table 1). The only dinucleotide repeat polymorphism reported is located in intron 3 [Granqvist et al., 1991]. We have identified two additional non-pathogenic changes upstream of exon 1, a 27 bp deletion (bases –48 to –22) and deletion of one CTG repeat unit immediately upstream of the $G_s\alpha$

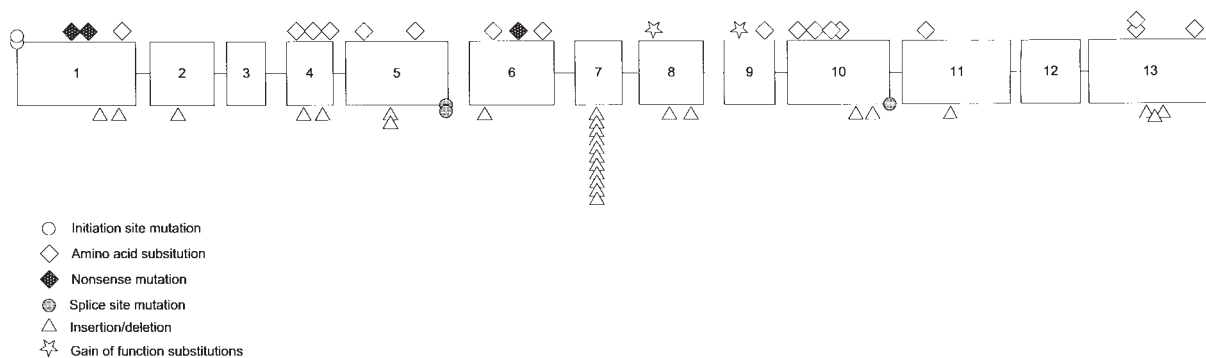


FIGURE 1. Schematic representation of mutations in GNAS1. Introns are not drawn to scale. The number of independent occurrences of each loss-of-function mutation is shown but for gain-of-function mutations, only the position is indicated. Polymorphic variants have been omitted.

translation initiation site. The 27 bp deletion was observed in a patient with AHO but not in her affected mother. Both were subsequently found to harbor a missense mutation (L132P) in exon 5. The 3 bp deletion was also identified in a patient with significant phenotypic features. It seems unlikely that this deletion would be pathogenic, but no other sequence variation has been identified in the patient to date. Each of these deletions has been observed in only one patient and are therefore not thought to represent common variants.

DIAGNOSTIC RELEVANCE

Detection of the specific activating mutations of $G_s\alpha$ can provide confirmation of a diagnosis of McCune-Albright syndrome. However, the mutations are typically present at a low level in lymphocytes while the affected tissues that harbor higher levels may not be routinely available for analysis. The use of mutation detection in a diagnostic service context therefore has limitations and requires highly sensitive techniques.

Mutation scanning to identify inactivating mutations in patients with suspected AHO has greater utility. AHO can be difficult to diagnose for a number of reasons. Clinically, the phenotype can show considerable variability [Wilson and Trembath, 1994]. Measurement of the level of $G_s\alpha$ activity is the most direct way of identifying patients with a GNAS1 mutation, but this assay is not routinely available in a service setting. Similarly, the lack of commercially available parathyroid hormone limits the ability to carry out PTH infusion testing to confirm the presence of hormone resistance. Mutation analysis of GNAS1 is therefore increasingly sought as a means of confirming a diagnosis of AHO and facilitating genetic counseling in affected families. In a study of patients in whom a 50% reduction in $G_s\alpha$ activity was confirmed, mutations were identified in approximately 80% of cases [Oude Luttikhuis, 1996]. Mutation scanning within a service setting is therefore both feasible and useful, but the absence of an identifiable mutation does not exclude a diagnosis of AHO.

FUTURE PROSPECTS

Approximately 20% of inactivating mutations remain undetected after mutation scanning with DGGE, SSCP, or sequencing. Future analysis must therefore be widened to look for whole exon deletions or other larger genomic rearrangements and for regulatory mutations.

On a functional level, many intriguing questions about this gene remain. Exons 2–13 of

GNAS1 are included in two additional overlapping transcripts, $XL\alpha_s$ and NESP55. Each has a distinct first exon. NESP55 is maternally expressed and exons 2–13 are probably not translated [Hayward et al., 1998b], whereas the $XL\alpha_s$ transcript is paternally expressed and exons 2–13 are in frame [Hayward et al., 1998a]. Paternally inherited mutations within exons 2–13 of GNAS1 may therefore affect the function of $XL\alpha_s$ as well as $G_s\alpha$. To add further complexity, an imprinted antisense transcript spanning the NESP55 region recently has been reported [Hayward and Bonthron, 2000] and the presence of further antisense transcripts is suggested by studies of the mouse *Gnas* gene cluster.

Mutations in exon 1 of GNAS1 can give rise to a classical AHO phenotype indistinguishable from that observed with mutations elsewhere in the gene. Since this exon is unique to the $G_s\alpha$ transcripts, NESP55 and $XL\alpha_s$ are not implicated in the primary AHO phenotype. However, investigation of possible interactions between these overlapping sense and antisense transcripts may shed light on the molecular basis of the strong parent-of-origin effect associated with mutations of GNAS1.

ACKNOWLEDGMENTS

The authors thank the many clinicians who have referred patient samples for mutation analysis. This work was supported by a grant from the Research Trust for Metabolic Diseases of Childhood (to R.C.T.).

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